WHAT IS CLAIMED IS:

1. A method for detecting a difference in the concentration of a protein present in a first sample and in a second sample, each sample comprising a plurality of proteins, the method comprising:

covalently attaching a first isotopic variant of a chemical moiety to a protein in the first sample to yield at least one first isotopically labeled protein;

covalently attaching a second isotopic variant of the chemical moiety to a protein in the second sample to yield at least one second isotopically labeled protein, wherein the first and second isotopically labeled proteins are chemically equivalent yet isotopically distinct;

mixing at least portions of the first and second samples to yield a combined sample; and

subjecting the combined sample to mass spectrometric analysis to determine a normalized isotope ratio characterizing proteins whose concentration is the same in the first and second samples and an isotope ratio of the first and second isotopically labeled proteins, wherein a difference in the isotope ratio of the first and second isotopically labeled proteins and the normalized isotope ratio is indicative of a difference in concentration of the protein in the first and second samples

2. The method of claim 1 further comprising fractionating the combined sample to yield at least one fraction comprising the isotopically labeled first and second proteins prior to determining the isotope ratios.

3. The method of claim 2 wherein fractionating the combined sample comprises subjecting the proteins to multidimensional chromatography, two-dimensional electrophoresis, affinity fractionation, or a combination thereof.

4. A method for detecting a difference in the concentration of a protein present in a first sample and in a second sample, each sample comprising a plurality of proteins, the method comprising:

covalently attaching a first isotopic variant of a chemical moiety to a protein in the first sample to yield at least one first isotopically labeled protein;

covalently attaching a second isotopic variant of the chemical moiety to a protein in the second sample to yield at least one second isotopically labeled protein, wherein the first and second isotopically labeled proteins are chemically equivalent yet isotopically distinct;

fragmenting proteins in the first and second samples to yield first and second isotopically labeled peptides in the first and second samples, respectively;

mixing at least portions of the first and second samples to yield a combined sample, wherein mixing is performed before or after fragmentation; and

subjecting the combined sample to mass spectrometric analysis to determine a normalized isotope ratio characterizing peptides derived from proteins whose concentration is the same in the first and second samples and an isotope ratio of the first and second isotopically labeled peptides, wherein a difference in the isotope ratio of the first and second isotopically labeled peptides and the normalized isotope ratio is indicative of a difference in concentration in the first and second samples of a protein derived from the peptide.

5. A method for detecting a difference in the concentration of a protein present in a first sample and in a second sample, each sample comprising a plurality of proteins, the method comprising:

fragmenting proteins in the first and second samples to yield at least one peptide in each sample;

covalently attaching a first isotopic variant of a chemical moiety to a peptide in the first sample to yield at least one first isotopically labeled peptide;

covalently attaching a second isotopic variant of the chemical moiety to a peptide in the second sample to yield at least one second isotopically labeled peptide, wherein the first and second isotopically labeled peptides are chemically equivalent yet isotopically distinct;

mixing at least portions of the first and second samples to yield a combined sample; and

subjecting the combined sample to mass spectrometric analysis to determine a normalized isotope ratio characterizing peptides derived from proteins whose concentration is the same in the first and second samples and an isotope ratio of the first and second isotopically labeled peptides, wherein a difference in the isotope ratio of the first and second isotopically labeled peptides and the normalized isotope ratio is indicative of a difference in concentration in the first and second samples of a protein derived from the peptide.

6. A method for detecting a difference in the concentration of a protein originally present in a first sample and in a second sample, each sample comprising a plurality of peptides derived from fragmentation of proteins originally present in the sample, the method comprising:

covalently attaching a first isotopic variant of a chemical moiety to a peptide in the first sample to yield at least one first isotopically labeled peptide;

covalently attaching a second isotopic variant of the chemical moiety to a peptide in the second sample to yield at least one second isotopically labeled peptide, wherein the first and second isotopically labeled peptides are chemically equivalent yet isotopically distinct;

mixing at least portions of the first and second samples to yield a combined sample; and

subjecting the combined sample to mass spectrometric analysis to determine a normalized isotope ratio characterizing peptides derived from proteins whose concentration is the same in the first and second samples and an isotope ratio of the first and second isotopically labeled peptides, wherein a difference in the isotope ratio of the first and second isotopically labeled peptides and the normalized isotope ratio is indicative of a difference in concentration in the first and second samples of a protein derived from the peptide

7. The method of claim 6 wherein the first and second chemical moieties are attached to at least one amino group on peptides in the first and second samples.

8. The method of claim 6 wherein each member of at least one pair of chemically equivalent, isotopically distinct peptides comprises at least one affinity ligand, the method further comprising, prior to determining the isotope ratios, contacting the peptides with a capture moiety to select peptides comprising the at least one affinity ligand.

9. The method of claim 8 further comprising subjecting the selected peptides comprising the at least one affinity ligand to mass spectrometric analysis to detect at least one peptide; and identifying the protein from which the detected peptide was derived.

10. The method of claim 9 wherein the detected peptide is a signature peptide for a protein, the method further comprising determining the mass of the signature peptide and using the mass of the signature peptide to identify the protein from which the detected peptide was derived.

11. The method of claim 10 further comprising determining the amino acid sequence of the detected peptide and using the amino acid sequence of the detected peptide to identify the protein from which the detected peptide was derived.

12. The method of claim 8 further comprising subjecting the selected peptides comprising the at least one affinity ligand to mass spectrometric analysis to determine peak intensities; and quantitating isotope ratios from the peak intensities.

13. The method of claim 8 further comprising, prior to contacting the peptides with the capture moiety, covalently attaching at least one affinity ligand to at least one peptide derived from the fragmentation of the proteins.

14. The method of claim 5 further comprising, prior to fragmenting the proteins, covalently attaching at least one affinity ligand to at least one protein in the sample.

15. The method of claim 5 further comprising reducing and alkylating the proteins with an alkylating agent prior to fragmenting the proteins.

16. The method of claim 15 wherein the at least one affinity ligand is covalently attached to the alkylating agent.

17. The method of claim 8 wherein the at least one affinity ligand is covalently attached to an amino acid of the peptide selected from the group consisting of cysteine, tyrosine, tryptophan, histidine and methionine.

18. The method of claim 8 wherein the affinity ligand comprises a moiety selected from the group consisting of a peptide antigen, a polyhistidine, a biotin, a dinitrophenol, an oligonucleotide and a peptide nucleic acid.

19. The method of claim 8 wherein at least one peptide comprises an endogenous affinity ligand.

20. The method of claim 19 wherein the endogenous affinity ligand comprises a moiety selected from the group consisting of a cysteine, a histidine, a phosphate group, a carbohydrate moiety and an antigenic amino acid sequence.

21. The method of claim 10 comprising attaching a plurality of affinity ligands, each to at least one protein or peptide, and contacting the peptides with a plurality of capture moieties to select peptides comprising at least one affinity ligand.

22. The method of claim 5 wherein the proteins are fragmented using an enzyme selected from the group consisting of trypsin, chymotrypsin, gluc-C, endo lys-C, pepsin, papain, proteinase K, carboxypeptidase, calpain and subtilisin.

23. The method of claim 6 further comprising fractionating the peptides prior to determining the isotope ratios.

24. The method of claim 23 wherein fractionating the peptides comprises subjecting the peptides to at least one separation technique selected from the group consisting of reversed phase chromatography, ion exchange chromatography, hydrophobic interaction chromatography and size exclusion chromatography, capillary gel electrophoresis, capillary zone electrophoresis,

and capillary electrochromatography, capillary isoelectric focusing, immobilized metal affinity chromatography and affinity electrophoresis.

25. The method of claim 6 wherein the sample comprises at least about 100 proteins.

26. The method of claim 10 wherein using the mass of the signature peptide to identify the protein from which the signature peptide was derived comprises comparing the mass of the signature peptide with the masses of reference peptides derived from putative proteolytic cleavage of a plurality of reference proteins in a database, wherein at least one reference peptide comprises at least one affinity ligand.

27. The method of claim 26 wherein peptides derived from proteolytic cleavage of the plurality of reference proteins are, prior to comparing the mass of the signature peptide with the masses of the reference peptides, computationally selected to exclude reference peptides that do not contain an amino acid upon which the affinity selection is based.

28. The method of claim 6 wherein the protein is in regulatory flux in response to a stimulus, and wherein the first sample is obtained from the biological environment before application of the stimulus and the second sample is obtained from the biological environment after application of the stimulus.

29. The method of claims 6 wherein the first and second samples are obtained from different organisms, cells, organs, tissues or bodily fluids, the method further comprising determining differences in concentration of at least one protein in the organisms, cells, organs, tissues or bodily fluids from which the samples were obtained.

30. The method of claim 1 further comprising identifying a plurality of isotopically labeled proteins having substantially the same isotope ratios, wherein the existence of said plurality of isotopically labeled proteins is indicative that the proteins are co-regulated.

31. The method of claims 6 further comprising identifying a plurality of isotopically labeled peptides having substantially the same isotope ratios, wherein the existence of said plurality of isotopically labeled peptides is indicative that the peptides are derived from the same protein, or from proteins that are co-regulated.

32. The method of claims 6 wherein the samples are obtained from a biological environment, and wherein the first sample is obtained from the biological environment before application of a stimulus and the second sample is obtained from the biological environment after application of the stimulus.

33. A method for detecting a difference in the concentration of a protein present in a first sample and in a second sample, each sample comprising a plurality of proteins, the method comprising:

providing a first and second sample, wherein the first sample is obtained from a biological environment prior to the application of a stimulus and the second sample is obtained from the biological environment after the application of the stimulus;

fragmenting proteins in the first and second samples to yield peptides;

contacting the peptides in the first sample with a first acylating agent comprising a first isotope to yield at least one first isotopically labeled peptide;

contacting the peptides in the second sample with a second acylating agent comprising a second isotope to yield at least one second isotopically

labeled peptide, wherein the first and second isotopically labeled peptides are chemically equivalent yet isotopically distinct;

mixing at least portions of the first and second samples to yield a combined sample;

contacting peptides in the combined sample with a capture moiety to select at least one pair of chemically equivalent isotopically distinct peptides comprising at least one affinity ligand;

fractionating the selected peptides yield a plurality of peptide fractions amenable to mass spectrometric isotope ratio analysis;

subjecting the combined sample to mass spectrometric analysis to determine a normalized isotope ratio characterizing peptides derived from proteins whose concentration is the same in the first and second samples and an isotope ratio of the first and second isotopically labeled peptides, wherein a difference in the isotope ratio of the first and second isotopically labeled peptides and the normalized isotope ratio is indicative of a difference in concentration in the first and second samples of a protein derived from the peptide; and

identifying the protein from which the peptide was derived.

34. A method for determining whether a protein is present in a one sample but not in another sample, each sample comprising a plurality of proteins, the method comprising:

providing a first and second sample, wherein the first sample is obtained from a biological environment prior to the application of a stimulus and the second sample is obtained from the biological environment after the application of the stimulus;

fragmenting proteins in the first and second samples to yield peptides;

partitioning the first sample into a first subsample and a second subsample;

contacting the peptides in the first subsample with a first acylating agent comprising a first isotope;

contacting the peptides in the second subsample with a second acylating agent comprising a second isotope;

contacting the peptides in the second sample with a third acylating agent comprising a third isotope, wherein the first, second and third acylating agents are chemically equivalent yet isotopically distinct;

mixing at least portions of the first and second subsamples and the second sample to yield a combined sample;

fractionating the peptides in the combined sample to yield a plurality of peptide fractions amenable to mass spectrometric isotope ratio analysis; and

subjecting at least one peptide fraction to mass spectrometric isotope ratio analysis, wherein the presence of a doublet indicates the absence of the protein in the second sample and the presence of a single peak indicates the absence of the protein in the first sample.

35. A method for analyzing differences in protein content among plural protein samples, the method comprising:

fragmenting at least a first protein sample and a second protein sample to produce a first peptide pool and a second peptide pool;

isotopically labeling at least a portion of the peptides in at least one of the pools so as to permit resolution of otherwise identical peptides in the first and second peptide pools by mass analysis;

contacting peptides from at least a portion of both of the peptide pools with a capture moiety to yield affinity-selected peptides comprising an affinity ligand, wherein the capture moiety selects for the affinity ligand; and

analyzing the affinity-selected peptides by mass spectrometry to determine one or more differences between the first and second samples.

36. The method of claim 35 wherein the labeling step comprises labeling at least one of the N-termini or the C-termini of the portion of the peptides.

37. The method of claim 36 wherein the labeling step comprises labeling both the N-termini and the C-termini of the portion of the peptides.

38. The method of claim 36 wherein the affinity ligand is an endogenous affinity ligand.

39. The method of claim 35 wherein the affinity ligand does not comprise the isotope label.

40. The method of claim 35 further comprising combining at least portions of the first and second pools after the labeling step but prior to the analyzing step.

41. The method of claim 35 wherein the affinity ligand is endogenous.

42. The method of claim 41 wherein the endogenous affinity ligand comprises an antigen.

43. The method of claim 42 wherein the affinity ligand comprises at least one antigen selected from the group consisting of a sugar, a lipid, a glycolipid and a peptide.

44. The method of claim 35 further comprising chemically coupling the affinity ligand to peptides.

45. The method of claim 35 further comprising reducing and alkylating the protein samples prior to the fragmenting step.

46. The method of claim 35 wherein the affinity-selected peptides comprise at least one low abundance amino acid selected from the group consisting of cysteine, tryptophan, histidine, methionine and tyrosine.

47. The method of claim 35 wherein the affinity-selected peptides comprise at least one phosphate group.

48. The method of claim 35 wherein the affinity-selected peptides comprise at least one oligosaccharide.

49. The method of claim 35 further comprising, prior to the analysis step, contacting the affinity-selected peptides with a second capture moiety to yield a subset of affinity-selected peptides comprising a second affinity ligand, wherein the capture moiety selects for the second affinity ligand.

50. The method of claim 49 wherein the second affinity ligand is an endogenous ligand.

51. The method of claim 50 wherein the first affinity ligand comprises the isotope label.

52 The method of claim 35 further comprising fractionating the affinity-selected peptides prior to analysis.

53. The method of claim 52 wherein the fractionation technique is selected from the group consisting of reversed phase chromatography, ion

exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, capillary gel electrophoresis, capillary zone electrophoresis and capillary electrochromatography, capillary isoelectric focusing, immobilized metal affinity chromatography and affinity electrophoresis.

54. The method of claim 35 further comprising fractionating the peptides subsequent to the contacting step to produce a second subset of peptides for mass spectrometric analysis.

55. The method of claim 35 wherein the mass spectrometric analysis is selected from the group consisting of matrix assisted laser desorption ionization (MALDI), electrospray ionization (ESI), fast atom bombardment (FAB), electron impact ionization, atmospheric pressure chemical ionization (APCI), time-of-flight (TOF), quadrapole, ion trap, magnetic sector, ion cyclotron resonance mass, or combinations thereof.

56. The method of claim 35 wherein the labeling step comprises labeling the first peptide pool with a first isotopic variant of a chemical moiety and the second peptide pool with a second isotopic variant of the chemical moiety to yield peptides in the first and second pools that are chemically equivalent but isotopically distinct; and wherein the analyzing step comprises analyzing the first sample and second samples by mass spectrometry; and comparing the mass spectrometry of the first and second samples.

57. The method of claim 56 wherein the analyzing step further comprises:

generating a first isotope ratio for the samples labeled with the first isotopic variant;

generating a second isotope ratio for the samples labeled with the second isotopic variant;

comparing the first isotope label ratio with the second isotope label ratio, wherein a difference between the first isotope label ratio with the second isotope label ratio is indicative of a difference in the relative concentration of the labeled peptides in the first and second sample.

58. The method of claim 57 wherein the first and second samples are combined prior to the analyzing step.

59. A method for quantifying a peptide comprising:

subjecting a sample comprising isotopically labeled isobaric peptides to mass spectrometric analysis to yield fragment ions, wherein at least two of the fragment ions are isotopically labeled and differ in mass with respect to each other; and

determining the isotope ratio of the at least two fragment ions, wherein the isotope ratio is indicative of the relative quantities of the isobaric peptides in the sample.

60. A method for quantifying a peptide comprising:

subjecting a sample comprising isotopically labeled peptides to a first mass spectrometric analysis to identify a plurality of isobaric peptides;

subjecting the plurality of isotopically labeled isobaric peptides to a second mass spectrometric analysis to yield fragment ions, wherein at least two of the fragment ions are isotopically labeled and differ in mass with respect to each other; and

determining the isotope ratio of the at least two fragment ions, wherein the isotope ratio is indicative of the relative quantities of the isobaric peptides in the sample.

61. A method for quantifying a peptide comprising:

subjecting a sample comprising isotopically labeled peptides to a first mass spectrometric analysis to identify a plurality of peptides whose masses overlap;

subjecting the plurality of isotopically labeled isobaric peptides to a second mass spectrometric analysis to yield fragment ions, wherein at least two of the fragment ions are isotopically labeled and differ in mass with respect to each other; and

determining the isotope ratio of the at least two fragment ions, wherein the isotope ratio is indicative of the relative quantities of the isobaric peptides in the sample.

62. The method of claim 61 wherein labeled and unlabeled forms of at least one peptide are present in the second dimension of mass spectrometry.

63. A method for identifying a protein in a sample comprising a plurality of proteins, the method comprising:

providing peptides derived from fragmentation of proteins in a sample comprising a plurality of proteins, wherein at least one peptide derived from the protein to be identified comprises at least one affinity ligand;

contacting the peptides with a capture moiety to select peptides comprising.the affinity ligand;

fractionating the selected peptides to yield a plurality of peptide fractions;

subjecting the peptides in at least one peptide fraction to mass spectrometric analysis to detect at least one peptide derived from the protein to be identified; and

identifying the protein from which the detected peptide was derived.

64. The method of claim 63 wherein the detected peptide is a signature peptide of the protein to be identified, the method further comprising determining the mass of the signature peptide and using the mass of the signature peptide to identify the protein from which the detected peptide was derived.

65. The method of claim 63 further comprising determining the amino acid sequence of the detected peptide and using the amino acid sequence of the detected peptide to identify the protein from which the detected peptide was derived.

66. The method of claim 63 further comprising, prior to contacting the peptides with the capture moiety, covalently attaching at least one affinity ligand to at least one peptide derived from the fragmentation of the proteins.

67. The method of claim 63 further comprising, prior to fragmenting the proteins, covalently attaching at least one affinity ligand to at least one protein in the sample.

68. The method of claim 63 further comprising reducing and alkylating the proteins with an alkylating agent prior to fragmenting the proteins.

69. The method of claim 68 wherein the at least one affinity ligand is covalently attached to the alkylating agent.

70. The method of claim 63 wherein the at least one affinity ligand is covalently attached to an amino acid of the peptide selected from the group consisting of cysteine, tyrosine, tryptophan, histidine and methionine.

71. The method of claim 63 wherein the affinity ligand comprises a moiety selected from the group consisting of a peptide antigen, a polyhistidine, a biotin, a dinitrophenol, an oligonucleotide and a peptide nucleic acid.

72. The method of claim 63 wherein at least one peptide comprises an endogenous affinity ligand.

73. The method of claim 72 wherein the endogenous affinity ligand comprises a phosphate group or a carbohydrate.

74. The method of claim 73 wherein the endogenous affinity ligand comprises a phosphate group, and wherein contacting the peptides with a capture moiety comprises contacting the peptides at acidic pH with a cationic support surface.

75. The method of claim 72 wherein the endogenous affinity ligand comprises a cysteine or a histidine.

76. The method of claim 72 wherein the endogenous affinity ligand comprises an antigenic amino acid sequence.

77. The method of claim 63 further comprising attaching a plurality of affinity ligands, each to at least one protein or peptide, and contacting the peptides with a plurality of capture moieties to select peptides comprising at least one affinity ligand.

78. The method of claim 63 further comprising fragmenting the proteins in the sample to yield the peptides.

79. The method of claim 78 wherein the proteins are fragmented using an enzyme selected from the group consisting of trypsin, chymotrypsin, gluc-C, endo lys-C, pepsin, papain, proteinase K, carboxypeptidase, calpain and subtilisin.

80. The method of claim 63 wherein fractionating the selected peptides comprises subjecting the selected peptides to at least one separation technique selected from the group consisting of reversed phase chromatography, ion exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, capillary gel electrophoresis, capillary zone electrophoresis and capillary electrochromatography, capillary isoelectric focusing, immobilized metal affinity chromatography and affinity electrophoresis.

81. The method of claim 63wherein the sample comprises at least about 100 proteins.

82. The method of claim 63 wherein using the mass of the signature peptide to identify the protein from which the signature peptide was derived comprises comparing the mass of the signature peptide with the masses of reference peptides derived from putative proteolytic cleavage of a plurality of reference proteins in a database, wherein at least one reference peptide comprises at least one affinity ligand.

83. The method of claim 82 wherein peptides derived from fragmentation of the plurality of reference proteins are, prior to comparing the mass of the signature peptide with the masses of the reference peptides, computationally selected to exclude reference peptides that do not contain an amino acid upon which the affinity selection is based.

84. A method for identifying a protein in a sample comprising a plurality of proteins, the method comprising:

providing peptides derived from fragmentation of proteins in a sample comprising a plurality of proteins, wherein at least one peptide comprises at least one affinity ligand;

contacting the peptides with a capture moiety to select peptides comprising the at least one affinity ligand;

determining the mass of at least one peptide comprising the at least one affinity ligand which is a signature peptide of the protein; and

using the mass of the signature peptide to identify the protein from which the signature peptide was derived.

85. A method for analyzing a protein in a sample comprising a plurality of proteins, the method comprising the steps of:

providing a sample comprising at least one protein comprising a signature peptide comprising an affinity ligand;

fragmenting the proteins in the sample to produce a peptide pool;

contacting peptides from at least a portion of the peptide pool with a capture moiety that selects for the affinity ligand to select peptides comprising the signature peptide, wherein the affinity ligand does not include an isotopic label; and

analyzing at least a portion of the peptide pool by mass spectroscopy.

86. The method of claim 85 wherein fragmenting the proteins comprises contacting the proteins with at least one of a chemical proteolytic agent, and enzymatic proteolytic agent and a mechanical proteolytic agent.

87. The method of claim 85 wherein the affinity ligand is endogenous to the signature peptides.

88. The method of claim 87 wherein the affinity ligand comprises an antigen.

89. The method of claim 88 wherein the affinity ligand comprises an antigen selected from the group consisting of a sugar, a lipids, a glycolipid, and a peptide.

90. The method of claim 85 wherein the affinity ligand comprises an exogenous affinity ligand.

91. The method of claim 85 wherein the protein sample is reduced and alkylated prior to fragmentation with an alkylating agent.

92. The method of claim 91 wherein the alkyating agent comprises the affinity ligand.

93. The method of claim 85 wherein the signature peptide comprises at least one low abundance amino acid selected from the group consisting of cysteine, tryptophan, histidine, methionine and tyrosine.

94. The method of claim 85 wherein the signature peptide comprises at least one phosphate group.

95. The method of claim 85 wherein the signature peptide comprises at least one oligosaccharide.

96. The method of claim 85 further comprising fractionating the affinity-selected peptides prior to analysis.

97. The method of claim 85 further comprising fractionating the peptides in the peptide pool prior to contacting the peptides with the capture moiety.

98. The method of claim 97 wherein the fractionation technique is selected from the group consisting of reversed phase chromatography, ion exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, capillary gel electrophoresis, capillary zone electrophoresis and capillary electrochromatography, capillary isoelectric focusing, immobilized metal affinity chromatography and affinity electrophoresis.

99. The method of claim 85 wherein the analyzing step comprises mass spectrometric analysis selected from the group consisting of matrix assisted laser desorption ionization (MALDI), electrospray ionization (ESI), fast atom bombardment (FAB), electron impact ionization, atmospheric pressure chemical ionization (APCI), time-of-flight (TOF), quadrapole, ion trap, magnetic sector, ion cyclotron resonance mass, or combinations thereof..